this approach was unlikely to produce any serious distortion of the location of substances in the cell:

> It may be assumed that the redistribution of substances in the cells frozen rapidly at a temperature of liquid air is only such as is determined by the formation of ice crystals. Apart from this pushing aside of the protoplasmic constituents by ice crystals, the distribution of protoplasm itself will remain for the most part unaltered.

In addition, freeze-drying would not remove the water soluble constituents of the cell. As they noted, the technique did run the risk of forming ice crystals that could dislocate other cell structures, but such artifacts were usually sufficiently gross that they were easily recognized. The disadvantages of freeze-drying were that it was more difficult to perform and produced specimens that were extremely fragile.

Comparison of micrographs of freeze-dried cells with chemically fixed ones indicated that freeze-drying did not produce significantly different results from ordinary chemical drying.[34] Fernández-Morán (1952), for example, explored whether freeze drying yielded superior images of nerve fibers. Previous studies using chemical fixation had detected thin filaments in nerve axons and had indicated the existence of concentric laminated structures formed by thin membranes on the sheath. Fernández-Morán indicated the factors that gave rise to his concerns about these micrographs: "since the preparation techniques employed involve fixation, dehydration and in many cases embedding and sectioning of the nerve fibres, the existence of these elements cannot be definitely established until the artefacts introduced by such manipulations have been adequately evaluated" (p. 282). In this case, he concluded, "*A comparative study of fresh frozen sections from the same nerve segment, which were subjected either to freezing-drying or to parallel osmium fixation, shows that the axon and sheath structures revealed in both are nearly identical*, but better preservation and contrast was achieved in the osmium fixed sections. *For routine examinations osmium fixation of the thin fresh frozen sections would therefore appear to the method of choice*" (p. 291).

leaves the surface for the highly evacuated vapor phase and is trapped in a liquid nitrogen trap or expelled through the pumping system. In the freeze-drying method the pumping is continued until all the water molecules have escaped in this way" (p. 206). The tissue was embedded in paraffin while still in vacuum, and then cut into section and mounted on slides.

[34] Some researchers concluded that, if anything, freeze-drying produced greater artifacts: "Electron microscope studies of tissue culture preparations have shown that freeze-drying gives an incorrect picture of the structure of protoplasm, and it is reported that the shrinkage and distortion of certain micro-organisms such as *Bacillus megatherium* are even more marked after freeze-drying than after air-drying" (Drummond, 1950, pp. 92–3).